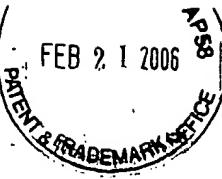


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T7 PROMOTER PRIMER

CTAATACGACTCACTATAGGG  
CTAATACGACTCACTATAGGG  
GATTATGCTGAGTGATATCCC

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cK XbaI PRIMER

CTGCAGGTGACTGTAGAGGGATCTACTAGT  
CATGCCTGCAGGTGACTCTAGAGGGATCTACTAGT  
GTACGGACGTCCAGCTGAGATCTCCTAGATGATCA

MUTAGENIC SITE XbaI

MUTAGENIC SITE

TTCTGTGCTCTATGGTACAGCAACCTCTGGGTATTCGGT  
AAGACACGGAGATAACCATGTCGTTGGAGACCCATAAGCCA  
CACGGAGATAACCATGACGTTGGAGACCCATA

S95C PRIMER

BamHI

CGTCGTGACTGGGAAAACC  
GCAGCACTGACCCCTTTGG  
GCAGCACTGACCCCTTTGG

U-19 PRIMER

pT7VICH255

1. PCR REACTION WITH T7 PRIMER AND S95C
2. PCR REACTION WITH KXbaI PRIMER AND S95C
3. MIX PRODUCTS FROM EACH AT 95°C/10 MIN COOL TO 55°C

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BamHI

4. FILL IN WITH POLYMERASE AND NUCLEOTIDES

BamHI

XbaI

5. AMPLIFY WITH PCR

BamHI

AND

BamHI

XbaI

**FIG. 1.**

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<u>GGTGCCTC</u> <u>XbaI</u>	GAG TCT GGG GGA GAC TCA GTG GAG TCT GGG GGA GAC TCA GTG AAG CCT GGA GGG TCC CTG AAA CTC GAC CTC AGA CCC CCT CTG AGTCAC TTC GGA CCT CCC AGG GAC TTT GAG Val Leu Val Glu Ser Gly Gly Asp Ser Val Lys Pro Gly GLY Ser Leu Lys Leu <b>FR1</b>
<u>TCC TGT GCA GCC TCT GGA TTC ACT TTA AGT GGT GAA ACC ATG TCT TGG GTT CGC CAG ACT</u> <u>AGG ACA CGT CGG AGA CCT AAG TGA AAT TCA CCA CTT TGG TAC AGA ACC CAA GCG GTC TGA</u> <u>Ser Cys Ala Ala Ser Gly Phe Thr Leu Ser Gly Glu Thr Mel Ser Trp Val Arg Gln Thr</u>	
<u>CCG GAG AAG AGG CTG GAG TGG GTC GCA ACC ACT CTT AGT GGT GGT TTC ACC TTC TAT</u> <u>GGC CTC TTC TCC GAC CTC ACC CAG CGT TGG TGA GAA TCA CCA CCA AAG TGG AAG ATA</u> <u>Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Thr Leu Ser Gly Gly Phe Thr Phe Tyr</u>	<b>CDR1</b> <b>FR2</b> <b>CDR2</b>
<u>TCA GCC AGT GTG AAG GGT CGT TTC ACC ATC TCC AGA GAC AAT GCC CAG AAC CTC TAT</u> <u>AGT CGG TCA CAC TTC CCA GCA AAG TGG TAG AGG TCT CTG TTA CGG GTC TTG GAG ATA</u> <u>Ser Ala Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Gln Asn Asn Leu Tyr</u>	<b>FR3</b>

**FIG. 2A.**

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85dA

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CTA CAA CTG AAT AGT CTG AGG TCT GAG GAC ACG GCC TTG TAT TTC TGT GCA AGT CAT CGG  
GAT GTT GAC TTA TCA GAC TCC AGA CTC CGG TGC CGG AAC ATA AAG ACA CGT TCA GTA GCC  
Leu Gln Leu Asn Ser Leu Arg Ser Glu Asp Thr Ala Leu Tyr Phe Cys Ala Ser His Arg

CDR3

TTT GTT CAC TGG GGC CAC GGG ACT CTG GTC ACT GTC TCT GCA GCC AAA ACG ACA CCC CCA  
AAA CAA GTG ACC CCG GTG CCC TGA GAC CAG TGA CAG AGA CGT CGG TTT TGC TGT GGG GGT  
Phe Val His Trp Gly His Gly Thr Val Leu Val Ser Ala Ala Lys Thr Thr Pro Pro

FR4

CCCCGGAGG  
Apal  
AGA CGT CGG TTT TGC TG  
TCT GCA GCC AAA ACG ACA CCC CCA  
CGT CGG TTT TGC TGT GGG GGT  
Ala Lys Thr Thr Pro Pro

CH1

FIG. 2B.

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SstI

CTCAGAGCTC

GCT	GTT	GTG	ACT	CAG	GAA	TCT	GCA	CTC	ACC	ACA	TCA	CCT	GGT	GAA	ACA	GTC	ACA	CTC	ACT	
CGA	CAA	CAC	TGA	GTC	CTT	AGA	CGT	GAG	TGG	TGT	AGT	CCA	CTT	TGT	CAG	TGT	GAG	TGA		
Ala	Val	Val	Thr	Gln	Glu	Ser	Ala	Ieu	Thr	Ser	Ala	Ieu	Thr	Gly	Glu	Thr	Val	Thr	Leu	Thr

FR1

TGT	CGC	TCA	AGT	ATT	GGG	GCT	GTT	ACA	ACT	AGT	AAC	TAT	GCC	AAC	TGG	GTC	CAA	GAA	AAA
ACA	GGG	AGT	TCA	TAA	CCC	CGA	CAA	TGT	TGA	TCA	TTG	ATA	CGG	TTG	ACC	CAG	GTC	CTT	TTT
Cys	Arg	Ser	Ser	Ile	Gly	Ala	Val	Thr	Thr	Ser	Asn	Tyr	Ala	Asn	Trp	Val	Gln	Glu	Lys

CDR1

CCA	GAT	CAT	TTA	TTC	ACT	GGT	CTA	ATA	GGT	ACC	AAT	AAC	CGG	GCT	CCG	GGT	GTT	CCT	
GGT	CTA	GTA	AAT	AAG	TGA	CCA	GAT	TAT	CCA	CCA	TGG	TAA	TTG	GCC	CGA	GTC	CCA	CAA	GGA
Pro	Asp	His	Leu	Phe	Thr	Gly	Ile	Gly	Ile	Gly	Thr	Asn	Asn	Arg	Ala	Pro	Gly	Val	Pro

CDR2

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GCC	AGA	TTC	TCA	GGC	TCC	CTG	ATT	GGA	GAC	AAG	GCT	GCC	CTC	ACC	ATC	ACA	GGG	GCA	CAG
CGG	TCT	AAG	AGT	CCG	AGG	GAC	TAA	CCT	CTG	TTC	CGA	CGG	GAG	TGG	TAG	TGT	CCC	CGT	GTC
Ala	Arg	Phe	Ser	Gly	Ser	Ile	Gly	Asp	Lys	Ala	Ala	Ieu	Thr	Ile	Thr	Ile	Gly	Ala	Gln

CDR3

**FIG. 3A.**

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ACT GAA GAT GAG GCA AGA TAT TTC TGT GCT CTA TCG TAC TGC AAC CTC TGG GTG TTC GGT  
TGA CTT CTA CTC CGT TCT ATA AAG ACA CGA GAT ACC ATG ACG TTG GAG ACC CAC AAG CCA  
Thr Glu Asp Glu Ala Arg Tyr Phe Cys Ala Leu Trp Tyr Cys Asn Leu Trp Val Phe Gly

FR4

GGA GGA ACC AAA CTG ACT GTC CTA AGC CAG CCC AAG TCT TCG CCA TCA GTC ACC CTG TTT  
CCT CCT TGG TTT GAC TGA CAG GAT TCG GTC GGG TTC AGA AGC GGT AGT CAG TGG GAC AAA  
Gly Gly Thr Lys Leu Thr Val Leu Ser Pro Lys Ser Ser Pro Val Thr Leu Phe

CDR3

FR4

BsiWI

CGTACGGCTC

CCG CCC TCC TCT GAA GAG CTA AGC TTG GGA ATC GGA TTC CCG GG  
GGC GGG AGG AGA CTT CTC GAT TCG AAC CCT TAG CCT AAG GGC CC  
Pro Pro Ser Ser Glu Glu Leu Ser Leu Gly Ile Gly Phe Pro Gly

FR4

CH1

FIG. 3B.

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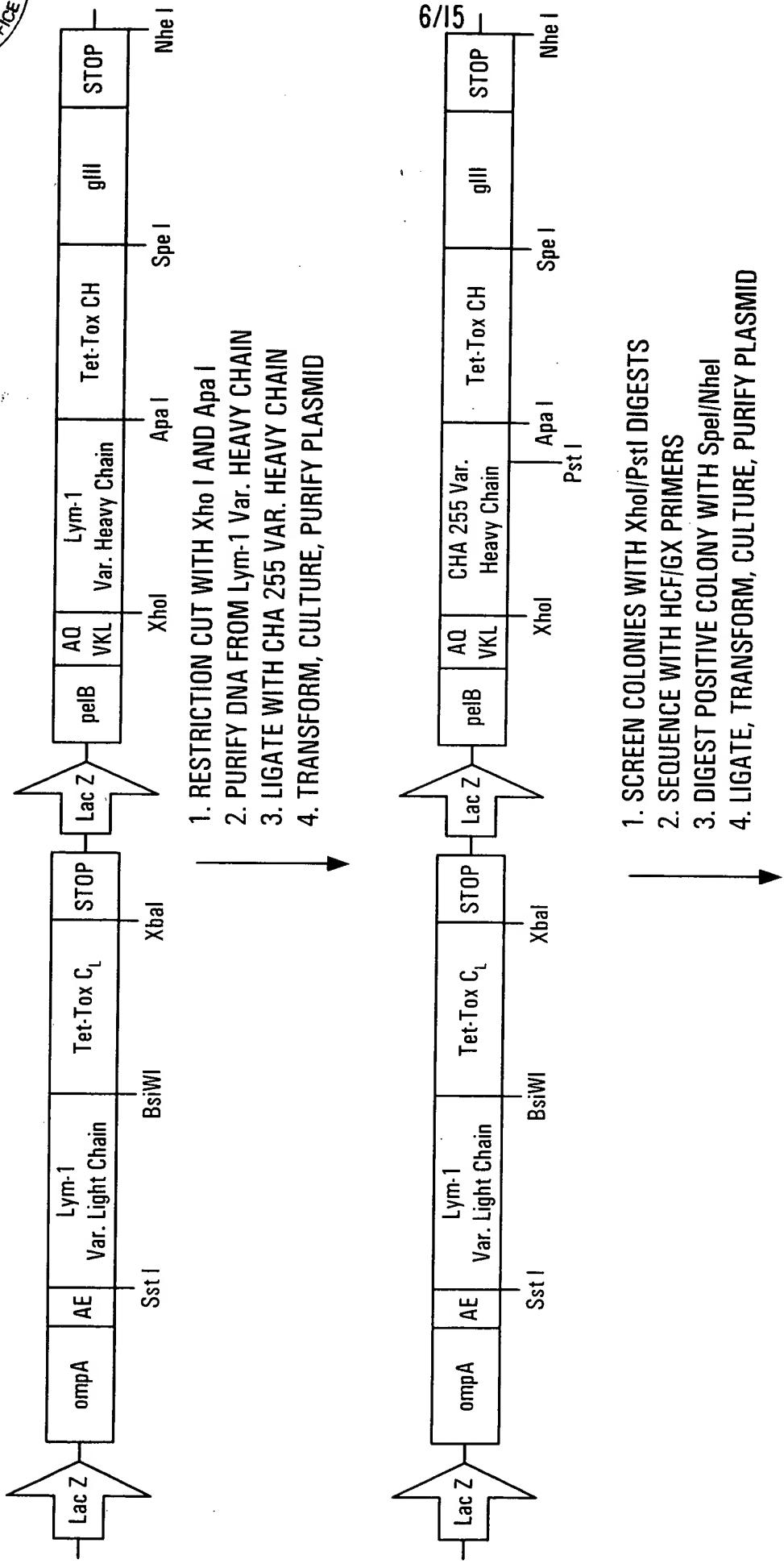
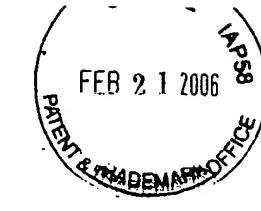


FIG. 4A.

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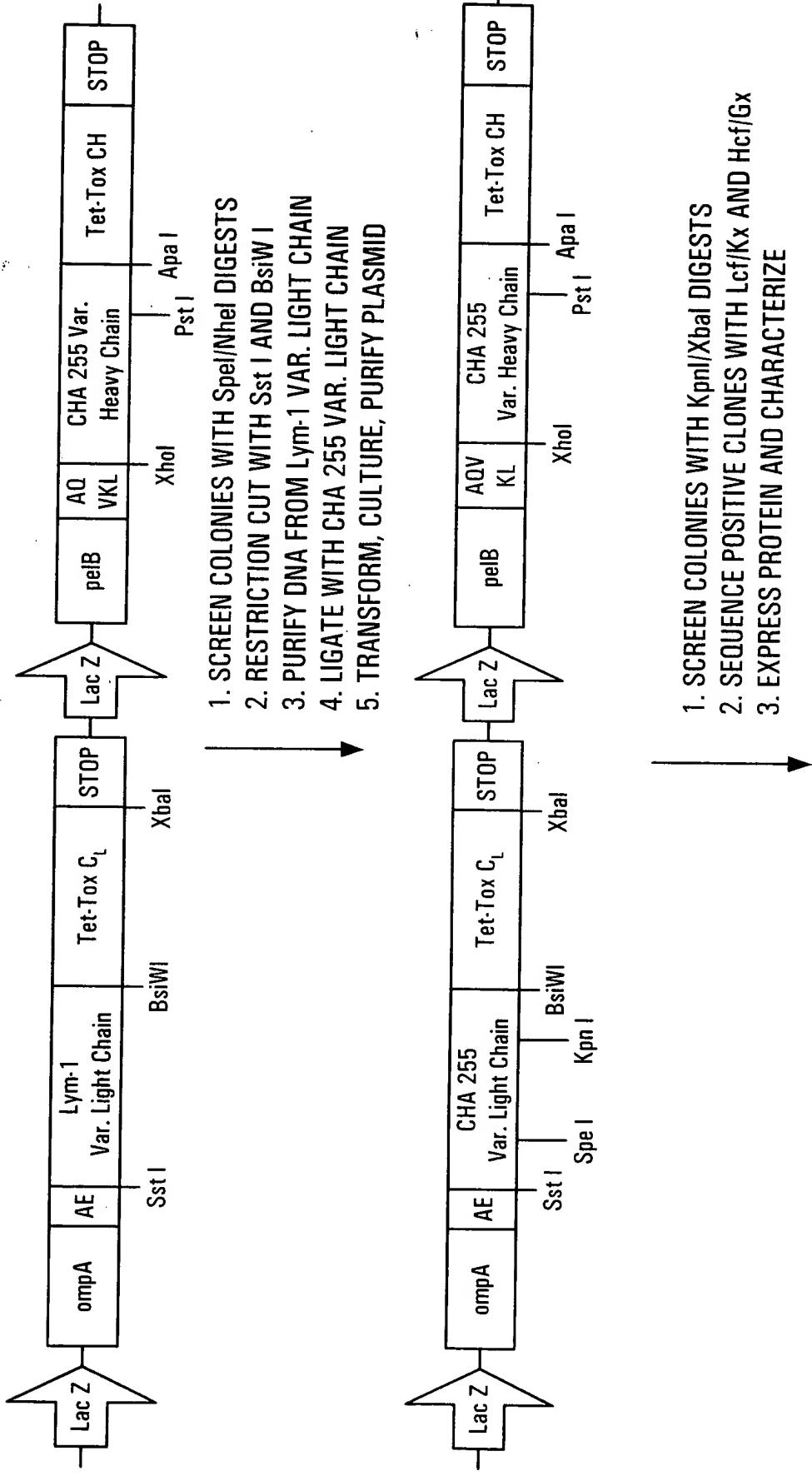
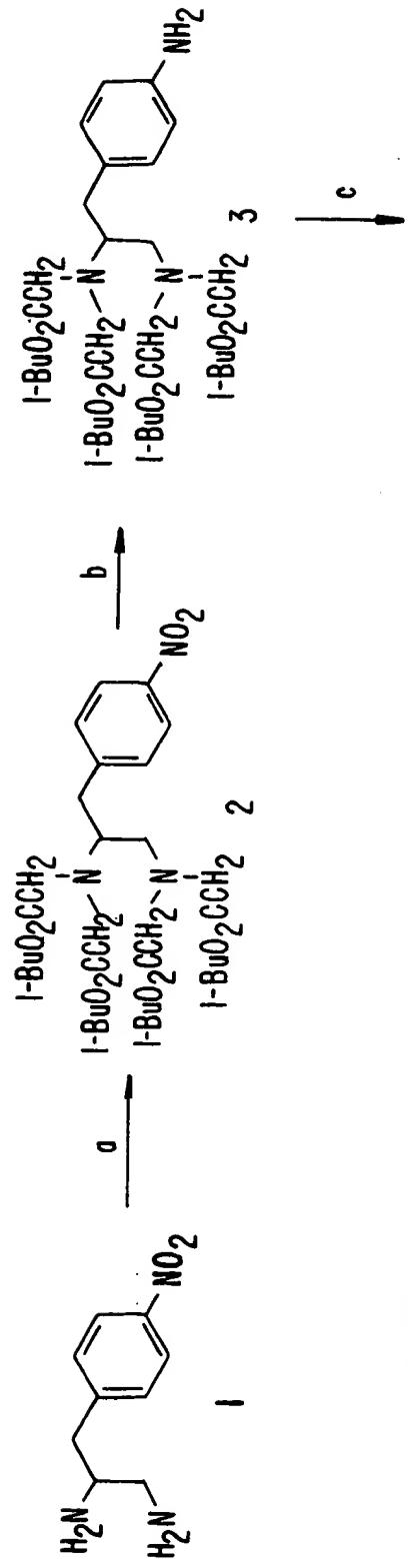


FIG. 4B.

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a.  $\text{BrCH}_2\text{CO}_2\text{t-Bu}$ , DIPEA, KI, DMF; b.  $\text{H}_2$ , Pd/C, MeOH; c. acryloyl chloride, DIPEA,  $\text{CH}_2\text{Cl}_2$ ; d. TFA

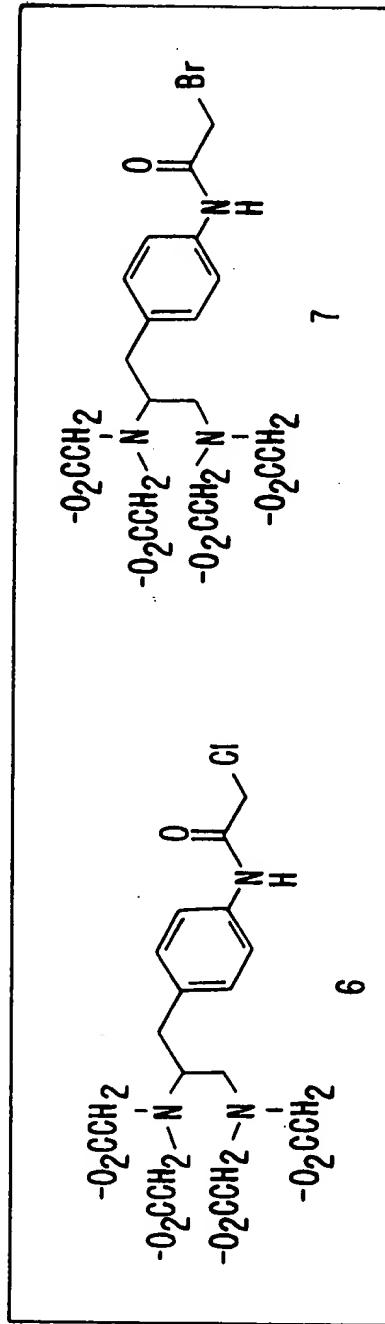


FIG. 5.



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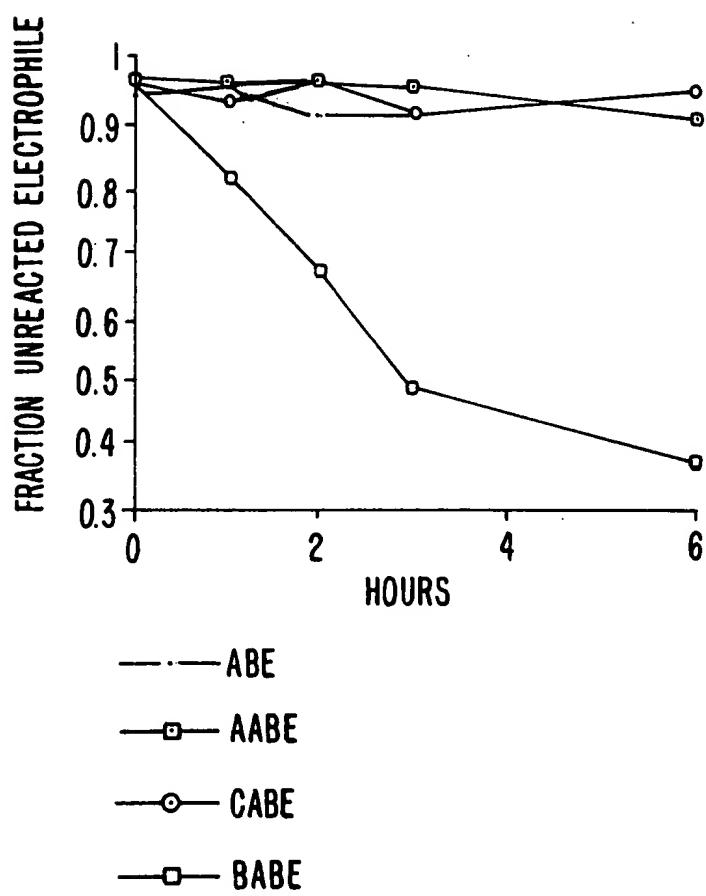


FIG. 6.

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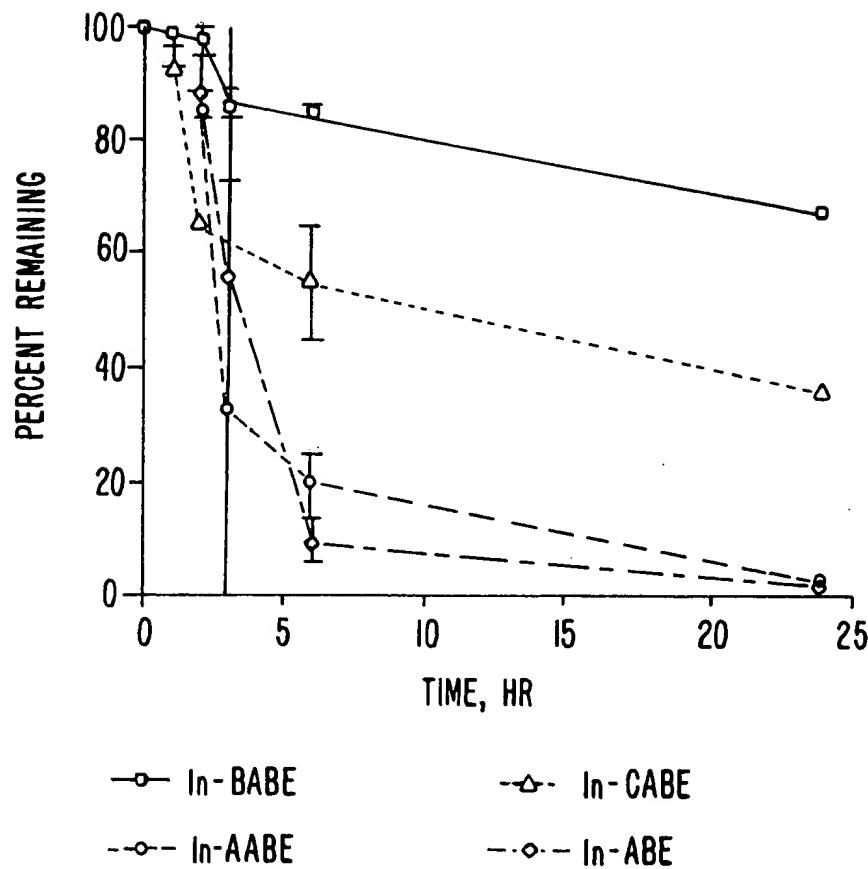


FIG. 7

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AGATCTGAAGTGACGCTGGAGTCTGGGGAGACTCAGTGAAGCCTGGAGGGTC  
CCTGAAACTCTCCTGTGCAGCCTCTGGATTCACTTAAGTGGTAAACCATGTCTG  
GGTCGCCAGACTCCGGAGAAGAGGCTGGAGTGGTOGCAACCACCTTAGTGGT  
GTGGTTCACCTCTATTCAAGCCAGTGTGAAGGGCTTCAACCACCTCCAGAGACA  
ATGCCAGAACAAACCTCTATCTACAACACTGAATAGTCTGAGGTCTGAGGACACGGCCT  
TGTATTCTGTGCAAGTCATCGGTTGTTCACTGGGCCACGGACTCTGGTCACTG  
TCTCTGCAGCCAAAACGAOACCCCCATCGGTCTTCCCCCTGGCACCCCTCCAAGA  
GCACCTCTGGGGCACAGCGGCCCTGGCTGCCTGGTCAAGGACTACTCCCCGAAC  
CGGTGACGGTGTGCGTGGAACTCAGGCCTGACCAGCGCGTGCACACCTCCGG  
CTGTCCTACAGTCCTCAAGACTCTACTTCCTCAGCAGCGTGGTACCGTGCCCTCA  
ACAGCTGGCACCCAGACCTACATGCAACGTGAATCACAAGCCCAGCAACACC  
AAGGTGGACAAGAAAGCAGAGCCAAATCTTGTGACAAATCTAGAGGGCCCTCGA  
AGGTAAAGCCTATCCCTAACCCCTCCTCGGTCTGATTCTACGCGTACCGGTATCA  
TCACCATCACCATTGA

**FIG. 8.**

AGATCTGCTGTTGACTCAGGAATCTGCACTCACCATCACCTGGTAAACAGTC  
ACACTCACTGTCGCTCAAGTATTGGGGCTTACAACACTAGTAACATGCCAAGTGG  
GTCCAAGAAAAACCAAGATCATTATTCACTGGTCTAATAGGTGGTACCAATAACCGG  
GCTCCGGGTGTTCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTC  
ACCATCACAGGGGCACAGACTGAAGATGAGGCAAGATATTCTGTGCTATGGTA  
CTCCTGCCTCTGGTRTTCGGTGGAGGAACCAAACACTGACTGTCCTAACCGWACKGT  
GGCTGCACCATCTGTCTTCATCTCCGCCATCTGATGAGCAGTTGAAATCTGGAAC  
TGCCTCTGTTGTGCGCTGCTGAATAACCTATCCCAGAGAGGCCAAAGTACAGTG  
GAAGGTGGATAACGCCCTCCAATCGGTAACCTCCAGGAGAGTGTACAGAGCAGG  
ACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCGTACGCTGAGCAAAGCAGAC  
TACGAGAAACACAAAGTCTACGCCCTGCGAAGTCACCCATCAGGGCTGAGYTYGCC  
CGTCACAAAGAGCTAACAGGGGAGAGTGTAA

**FIG. 9.**

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AGATCTGCTGTTGACTCAGGAATCTGCACTCACCATCACCTGGTAAACAGTC  
ACACTCACTGTCGCTCAAGTATTGGGGCTGTTACAACTAGTAACATGCCAAGTGG  
GTCCAAGAAAAACCAGATCATTATTCACTGGTCTAATAGGTGGTACCAATAACCGG  
GCTCCGGGTGTTCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTC  
ACCATCACAGGGGCACAGACTGAAGATGAGGCAAGATATTCTGTGCTATGGTA  
CTCCAACCTCTGGGTGTTCGGTGGAGGAACCAAACGTACTGTCTAACGCCAGCCC  
AGTCTTCGCCATCAGTCACCCCTGTTCCGCCCTCCTCTGAAGAGCTAACGCTGGGAA  
TCGGATTGCCGGGTGCTGTAATAACTCTATCCCAGAGAGGCCAAAGTACAGT  
GGAAGGTGGATAACGCCCTCCAATCGGGTAACCTCCCAGGAGAGTGTACAGAGCAG  
GACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGA  
CTACGAGAAACACAAAGTCTACGCCCTGCGAAGTCACCCATCAGGGCCTGAGYTYGC  
CCGTCACAAAGAGCTCAACAGGGAGAGTGTAA

**FIG. 10.**

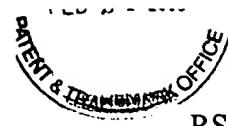
AGATCTGCTGTTGACTCAGGAATCTGCACTCACCATCACCTGGTAAACAGTC  
ACACTCACTGTCGCTCAAGTATTGGGGCTGTTACAACTAGTAACATGCCAAGTGG  
GTCCAAGAAAAACCAGATCATTATTCACTGGTCTAATAGGTGGTACCAATAACCGG  
GCTCCGGGTGTTCTGCCAGATTCTCAGGCTCCCTGATTGGAGACAAGGCTGCCCTC  
ACCATCACAGGGGCACAGACTGAAGATGAGGCAAGATATTCTGTGCTATGGTA  
CTCCAACCTCTGGGTGTTCGGTGGAGGAACCAAACGTACTGTCTAACGCCAGCCC  
AGTCTTCGCCATCAGTCACCCCTGTTCCGCCCTCCTCTGAAGAGCTAACGCTGGGAA  
TCGGATTCCCGGGTGCTGTAATAACTCTATCCCAGAGAGGCCAAAGTACAGT  
GGAAGGTGGATAACGCCCTCCAATCGGGTAACCTCCCAGGAGAGTGTACAGAGCAG  
GACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGA  
CTACGAGAAACACAAAGTCTACGCCCTGCGAAGTCACCCATCAGGGCCTGAGYTYGC  
CCGTCACAAAGAGCTCAACAGGGAGAGTGTAA

**FIG. 11.**

RSAVVTQESALTTSPGETVLTCSRSSIGAVTTSNYANWVQEKPDLHLFTGLIGGTNNR  
APGVPARFSGSLIGDKAALTITGAQTEDEARYFCALWYSCLWVFGGGTKLTVLSRTV  
AAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD  
SKDSTYSLSSLTLSKADYEHKVYACEVTHQGLSXPVTKSFNRGEC

**FIG. 12.**

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RSAVVTQESALTTSPGETVLTCSRSSIGAVTTSNYANWVQEKPDLHLFTGLIGGTNNR  
APGVPARFSGSLIGDKAALTITGAQTEDEARYFCALWYSNLWVFGGGTKLTVLSRTV  
AAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQD  
SKDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSXPVTKSFNRGEC

**FIG. 13.**

RSAVVTQESALTTSPGETVLTCSRSSIGAVTTSNYANWVQEKPDLHLFTGLIGGTNNR  
APGVPARFSGSLIGDKAALTITGAQTEDEARYFCALWYCNLWVFGGGTKLTVLSRTV  
AAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQD  
SKDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSXPVTKSFNRGEC

**FIG. 14.**

RSEVTLVEGRGDSVKPGGSLKLSCAASGFTLSGETMSWVRQTPEKRLEWVATTLSGG  
GFTFYSASVKGRFTISRDN  
AQNNLYLQLNLSLRSEDTALYFCASHRFVHWGHGLTVSAAKTPPSVFPLAPSSKS  
TSGGTAALGCLVKDYFPEP  
VTVSWNSGALTSGVHTFPAVLQSSRLYFLSSVVTVPFNSLGTQTYICNVNHKPSNTK  
VDKKAEPKSCDKSRGPFEG  
KPIPNPLLGLDSTRTGHHHHHH

**FIG. 15.**

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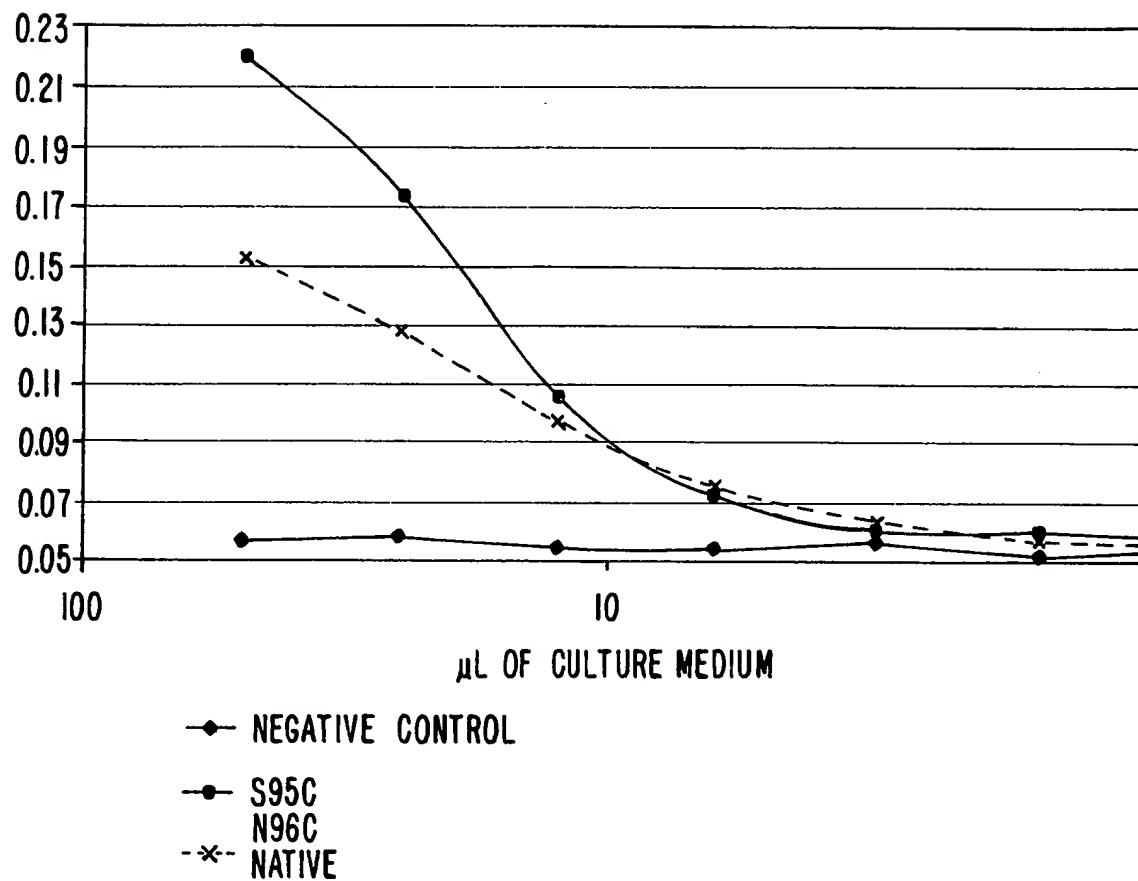
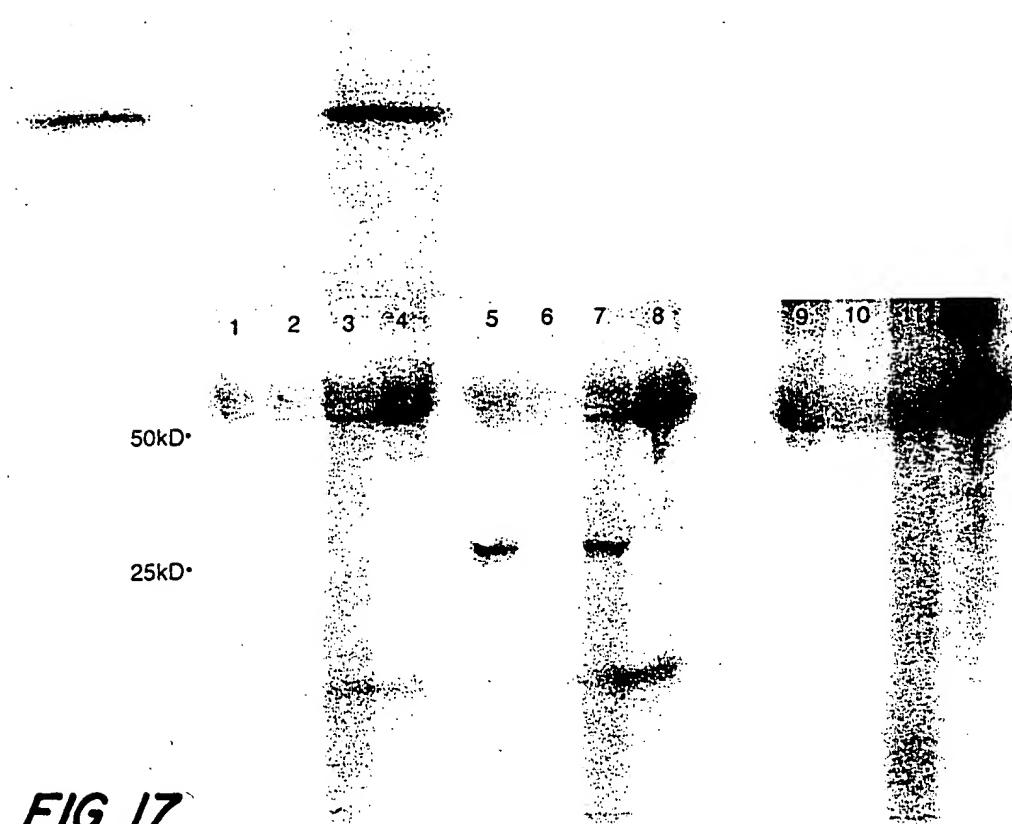
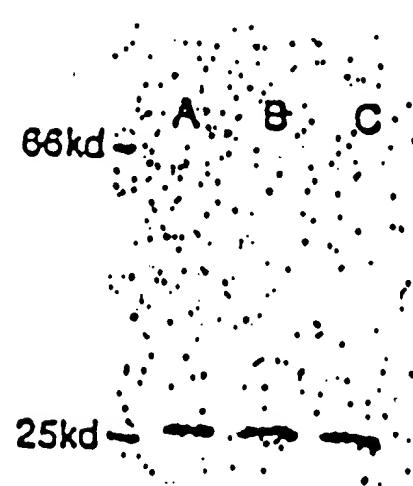


FIG. 16.

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**FIG. 17.**



**FIG. 18.**

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